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SHORT COMMUNICATION

Does the blood-sucking nematode *Ashworthius sidemi* (Trichostrongylidae) cause deterioration of blood parameters in European bison (*Bison bonasus*)?

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Abstract European bison (*Bison bonasus*), after extinction in the wild at the beginning of twentieth century, were reintroduced in over 30 free-ranging populations in central and eastern Europe. Major threats to this species include disease and parasites. In the last decades, the highly pathogenic blood-sucking nematode *Ashworthius sidemi* (Trichostrongylidae) has been found in high infection intensity and prevalence within the abomasa of free-living European bison. We investigated the impact of this invasion on blood parameters associated with the red blood cell system in 64 bison (53 free-living and 11 captive) which were culled in two neighbouring populations in north-eastern Poland between 2008 and 2015. We observed a significant decrease in red blood cell count, haemoglobin and haematocrit relative to the intensity of *A. sidemi* infection. Furthermore, the same parameters were significantly lower, and the percentage of reticulocytes was increased in highly infected free-living bison compared to captive individuals. Studies indicate the urgent need to change the conservation management of herds because traditional management that includes supplementary feeding causes a large concentration of bison in winter feeding areas. This causes an increase in parasitic load which ultimately impacts on their condition. The reservoir of this parasite in wildlife may also adversely affect livestock grazing on pastures used by bison.

Keywords Parasite invasion · Blood parameters · Conservation management · Health status

Introduction

The European bison (*Bison bonasus* L., 1758) is the largest terrestrial mammal in Europe. After extinction in the wild in 1919, it was restored from captive survivors and reintroduced to over 30 locations in eastern Europe (Krasińska and Krasiński 2013). In the wild, numbers now exceed 3300 of which almost 20 % occur in two populations situated in Białowieża and Knyszyn Forests in north-eastern Poland. Major threats to the species include diseases and parasites (Pucek et al. 2004). Bison have been parasitologically investigated and monitored with different intensity since their reintroduction to the wild in 1952 (Dróżdż 1961). A total of 88 species of parasites have already been discovered in bison, and there is an increasing trend in species richness as well as the prevalence and intensity of infections (Karbowiak et al. 2014a, 2014b). Recently, the blood-sucking nematode *Ashworthius sidemi* (Trichostrongylidae) has been assimilated within the abomasa of European bison. This parasite originated from Asian cervids (mainly the sika deer *Cervus nippon*). In Białowieża Primeval Forest (BPF), this parasite was first found in 2000 (Dróżdż et al. 2003). Between 2004 and 2012, all investigated European bison in BPF were infected (Kołodziej-Sobocińska et al. unpublished data). The maximal median rate was 8200 nematodes per animal in winter 2008/2009, and the highest number of *A. sidemi* recorded in one animal was 77,600, in 2011 (Demiaszkiewicz and Pyziel 2010; Kołodziej-Sobocińska et al. unpublished data). The parasite was also found in the neighbouring population in Knyszyn Forest (KF) in 2009. Since 2012, a drop of both infection intensity and prevalence has been observed in BPF

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while invasion is increasing in KF (Kołodziej-Sobocińska et al. unpublished data). The first evidence for the presence of *A. sidemi* in cattle (PCR analysis) confirms the possible transmission of the parasite from wildlife to livestock (Moskwa et al. 2015).

Blood-sucking parasites such as *A. sidemi* are strongly pathogenic. Histopathological examination of tissue from infected bison showed infiltrations of inflammatory cells in the walls of the abomasa and duodena (mainly lymphoid cells and eosinophils, hyperaemia, oedema, mucosal lesions and proliferation of lymphatic follicles) at various levels of intensity (Osińska et al. 2010). Invasion of a new parasite in the endangered population of bison may constitute a threat in light of the observed decline in their physical condition and increasingly female-biased calf sex ratios, which may be associated with an increase in parasitic load, especially invasive *A. sidemi* (Hayward et al. 2011). We hypothesise that the invasion of this blood-sucking nematode causes deterioration of blood parameters associated with the red blood cell system and results in a decline in bison condition and resistance.

Materials and methods

We investigated parasitic load and selected blood parameters associated with the red blood cell system in free-living and captive bison from two neighbouring (60 km apart) populations in north-eastern Poland between 2008 and 2015. Abomasa of bison were obtained from culled free-living ($N = 45$) and captive bison ($N = 11$). Bison were culled by rifle by Białowieża National Park staff (BF) or by hunters (KF). Permits for culling are issued each year by the Ministry of Environment and the General Directorate for Environmental Protection (Warsaw, Poland) and as approved by the bison management plan. The reason for the culling of captive bison was to reduce their numbers. Free-living bison were culled for the following reasons: reduction—37 %; injuries and limping—31 %; posthitis (necrotic inflammation of prepuce)—19 %, skin infections—5 %; road accidents—4 %; and poor condition—4 %. No animals were killed specifically for this study. The average body mass of culled males and females did not differ from the average body mass of the parent population (Krasińska and Krasiński 2002) indicating the representativeness of our sample. All blood samples were collected during the winter season which allowed us to omit seasonal variation of blood parameters. In winter time, both free-living and captive bison are supplementary fed so the possibility of nutritional differences is minimised. Captive bison are kept in semi-wild conditions in large enclosures (10–30 ha) and are regularly dewormed. The contents of the whole abomasa and a 1-m-long section of duodena from culled bison were examined by a standard scraping and sedimentation method (Demiaszkiewicz et al. 2013), and morphological identification of *A. sidemi* was carried out on the basis of

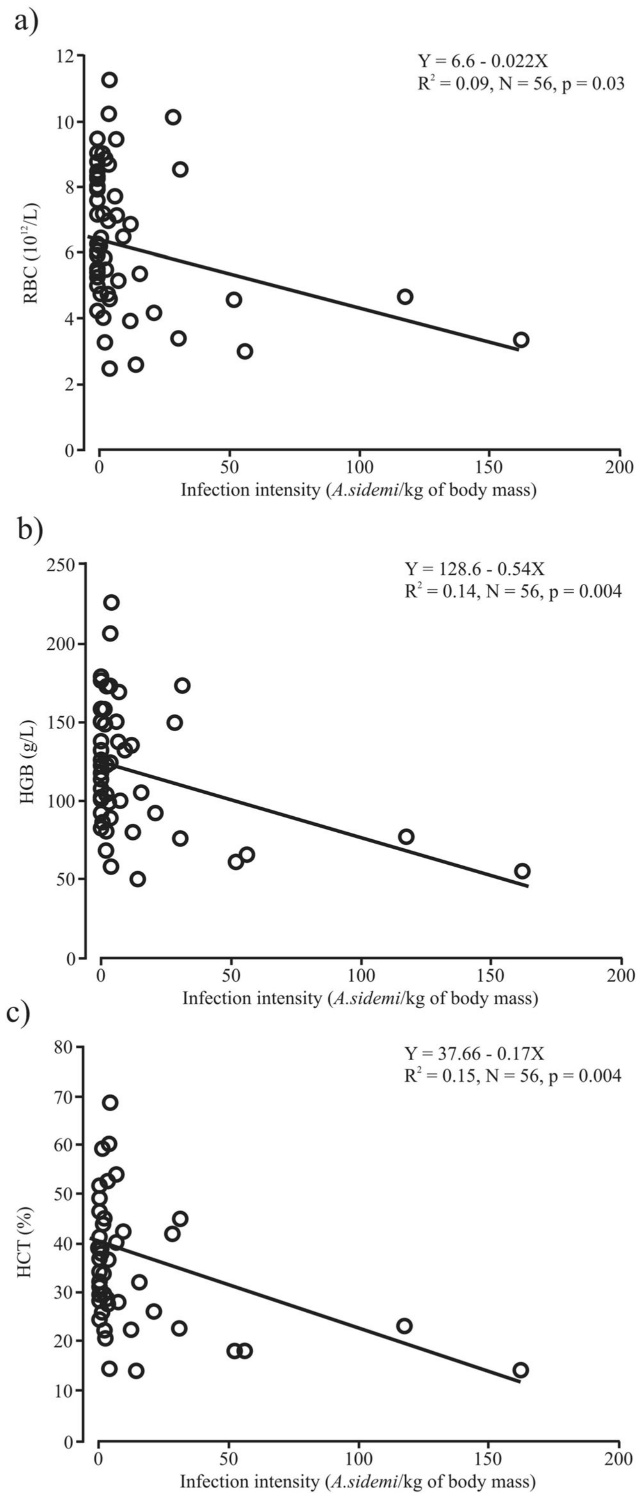

Fig. 1 The relationship between selected blood parameters and *A. sidemi* infection intensity in European bison in north-eastern Poland: **a**) red blood cells count (RBC), **b**) haemoglobin concentration (HGB), **c**) haematocrit value (HCT). Intensity of *A. sidemi* infection was recalculated per kilogramme of bison body mass to take into account age- and sex-related differences in animal size

Dróżdż et al. (1998). Blood analysis was performed on 64 blood samples collected from captive bison from the Bison Breeding Centre of the Białowieża National Park ($N = 11$) and from free-living bison ($N = 53$). For eight studied bison, we had no parasitological data, but these samples were included in the comparison between free-ranging and captive animals. To avoid differences in blood parameters associated with age and sex, juvenile bison up to 1 year old were excluded and both groups (captive and free-living animals) consisted of a similar number of males and females. Blood for haematology and serum for iron concentration detection was obtained from the jugular vein after a bison had been shot. Blood for haematology was collected in 10-ml tubes with ethylenediaminetetraacetic acid anticoagulant (EDTA), cooled and transported to laboratory where haematological analysis was performed the same day. Blood for serum biochemistry was collected in standard 10-ml serum-separating tubes containing particles which cause blood to clot quickly. Serum-separating tubes were then kept at room temperature for 1–2 h to ensure complete clotting. Serum was separated by double centrifugation at 1000×*g* for 10 min (Wołk and Józefczak 1988; Wołk and Krasińska 2004; Rostal et al. 2012). Analysis was performed on the automatic haematology analyser—BC-2800Vet and chemistry analyser—BS-120 (Mindray). The percentage of reticulocytes stained with Brilliant Cresyl Blue solution (MedLab Products) was determined in smears using a 100× microscope objective.

Results and discussion

We found that parameters associated with the red blood cell system such as red blood cell count (RBC), haemoglobin (HGB) and haematocrit (HCT) were negatively correlated with increasing intensity of *A. sidemi* infection in bison (Fig. 1). The thrombocyte (PLT) count, percentage of reticulocytes (RET) and iron concentration (Fe) were not dependent on *A. sidemi* infection intensity. A comparison of studied parameters between captive animals with low *A. sidemi* infection intensity and free-living bison with high *A. sidemi* infection intensity revealed significantly lower RBC, HGB and HCT values, and a higher percentage of RET in free-living bison (Table 1). Thrombocyte count and iron concentration were also lower in free-living bison but insignificantly so (see Table 1).

The present study has demonstrated for the first time that the invasive blood-sucking nematode *Ashworthius sidemi* is a cause of significant deterioration of blood parameters connected with the red blood system in bison. We revealed that *A. sidemi* infection occurring at a high infection rate and prevalence in free-living European bison results in a decrease of RBC, HGB and HCT measurements and an increase in RET (reticulocytosis). Reticulocytosis represents bone marrow compensation for haemolysis or blood loss (Doig 2012). An increased compensatory production of reticulocytes shows that infected bison can replace the loss of RBC. However, in severe *A. sidemi* infection, losses in RBC are probably too high: the compensation is not sufficient which can lead to deficiencies in parameters associated with the red blood cell system.

A high proportion of current bison populations are managed in forest habitats and are supplementary fed in winter to mitigate migration and reduce damage to farm crops and tree stands (Kerley et al. 2012). Ecological studies over recent years have shown the long-term effects of management practices on parasitic load in bison. A strong positive relationship was found between the winter densities of bison and the

Table 1 Comparison of blood-sucking nematode *A. sidemi* infection and selected blood parameters between captive and free-living European bison in north-eastern Poland

Mean ± SE (range)

Parameter	Captive	*N*	Free-living	*N*	Statistical analysis
A. sidemi prevalence (% of bison infected)	54.5	11	91.1	45	G test $G = 9.6$, $df = 1$, $p < 0.01$
Infection intensity (*A. sidemi*/animal)	77 ± 67 (0–750)	11	4144 ± 1101 (0–34,070)	45	Mann-Whitney test $U = 52$, $p < 0.0001$
RBC (10^{12}/L)	8.2 ± 0.2 (7.1–9.4)	11	5.9 ± 0.3 (1.9–11.4)	53	$U = 96$, $p = 0.0005$
HGB (g/L)	157 ± 5 (136–178)	11	115 ± 6 (34–226)	53	$U = 87$, $p = 0.0003$
HCT (%)	44.3 ± 1.7 (33.0–51.9)	11	33.6 ± 1.7 (9.6–68.7)	53	$U = 114$, $p = 0.002$
PLT (10^9/L)	278 ± 74 (91–979)	11	226 ± 26 (36–923)	53	$U = 242$, $p = 0.38$
RET[a] (%)	2.2 ± 0.1 (2.0–2.9)	7	5.3 ± 0.6 (1.3–13.6)	36	$U = 42$, $p = 0.006$
Fe[b] (μg/dL)	177 ± 12 (109–230)	11	147 ± 10 (16–280)	49	$U = 194$, $p = 0.15$

[a] Smaller sample size resulted from the later implementation of this method; for reticulocyte proportion studies, fresh blood samples are required so we could not add previously collected blood samples to the analysis

[b] Smaller sample size resulted from too small a volume of blood to allow serum separation for iron concentration analysis

intensity of *A. sidemi* infection (Radwan et al. 2010). Also, a higher prevalence of coccidia infection was found in both males and females in intensively fed herds which spent winter at high densities (Pyziel et al. 2011). This in turn may be inadvertently shaping the demography and the overall fitness of the European bison population (Hayward et al. 2011). It is also known that the high infection rate of pathogenic blood-sucking nematodes depends on management practices such as supplementary feeding in winter (Kołodziej-Sobocińska et al. unpublished data). Studies by Gatongi et al. (1998) on the blood-sucking nematode *Haemonchus contortus* revealed that high infection rates caused by extensive use of pastures by sheep results in life-threatening levels of anaemia and an associated potential for significant reductions in lamb survival. As a result, to avoid the consequences of nematode infections in ruminant livestock, sustainable nematode parasite control strategies are used such as grazing management and biological control (Waller 2006). Since *A. sidemi* has occurred in BPF, no haematophagous parasites, e.g. *Haemonchus contortus* have been found in bison abomasa (Demiaszkiewicz, pers. commun.). According to other blood-sucking parasites, hookworms have never been noted in European bison (Karbowiak et al. 2014a, 2014b). Although two species of blood-sucking nematodes were recorded in large intestine (*Bunostomum trigonocephalum* and *Trichuris ovis*) of bison but in low infection intensity (Demiaszkiewicz et al. 2012), so we can assume that the possibility of other haematophagous parasites influencing the observed pattern was not significant.

Our study provides direct evidence that a blood-sucking nematode invasion may cause deterioration of the blood parameters in wild animals and most likely affects their condition as a consequence. Taking into account the recent reports of the possible transmission of *A. sidemi* to cattle (Moskwa et al. 2015) and the increasing use of farmland by bison (Hofman-Kamińska and Kowalczyk 2012; Kowalczyk et al. 2013), continuous health monitoring and conservation management aimed at reducing parasite abundance in bison should be introduced to avoid serious consequences for this unique species and for cattle grazing on pastures used by bison.

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Compliance with ethical standards

Conflict of interest None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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